Defective Retrovirus-Like 30S RNA Species of Rat and Mouse Cells Are Infectious if Packaged by Type C Helper Virus

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RNA species with properties of defective retrovirus-like 30S RNA genomes have previously been detected in both rats and mice and in some rat and mouse retroviruses. Using cell lines which express high levels of this retrovirus-like RNA, we formed pseudotypes of the 30S RNAs with helper-independent type C viruses. A pseudotype virus complex containing a mouse 30S subunit was transmitted to rat cells, and a pseudotype virus complex containing a rat 30S subunit was transmitted to bat cells. In other transmission experiments, a rat 30S subunit was isolated in nonproducer bat cells without detectable expression of the helper-independent type C virus used to pseudotype it. The results provide further support for the retrovirus-like nature of the rat 30S subunit and provide evidence which supports the protovirus hypothesis proposed by Temin.

Harvey murine sarcoma virus (Ha-MSV; 8) and Kirsten murine sarcoma virus (Ki-MSV; 10) are replication-defective retroviruses with the genetic capacity to transform fibroblasts in cell culture. Both Ki-MSV and Ha-MSV have been shown to be mouse-rat hybrid viruses derived in an unknown manner by a recombinational event between a mouse type C virus and endogenous rat genetic information (18, 19). Recent studies have compared Ki-MSV and Ha-MSV by oligonucleotide fingerprinting and revealed that several kilobases of the rat genetic information in Ki-MSV are highly related to the rat genetic information in Ha-MSV and that these rat-derived genes are located at a similar place in the physical map of each virus (23).

Earlier reports have described properties of the endogenous rat genetic information homologous to the rat-derived genes found in Ki-MSV and Ha-MSV (1, 6, 15, 17, 20, 27). The endogenous rat information is present in multiple copies in the DNA of uninfected rat species. Some cell lines express high constitutive levels of RNA homologous to the rat sequences of Ki-MSV, and the predominant molecular RNA species in such cells sedimented at approximately 30S in sucrose gradients. Other cell lines which constitutively express low levels of such RNA could be induced with halogenated pyrimidines to express high levels of related RNA. The 30S RNA expressed constitutively in rat cells could be pseudotyped specifically by helper-independent type C retroviruses replicating in the cells, and the pseudotyped RNA could be reverse tran-

scribed to complementary DNA (cDNA) in endogenous reactions containing virions released from these cells. The 30S RNA present in such pseudotypes formed high-molecular-weight structures, presumably dimers, which sedimented at 60 to 70S in sucrose gradients (5). Thus, the DNA and RNA homologous to the Ki-MSV rat information had many properties in common with the genomes of known endogenous mammalian retroviruses. However, cell lines expressing this RNA did not form viral particles or as yet identifiable other macromolecular structures. The RNA was not homologous to helper-independent rat type C viruses, and cells expressing the RNA did not synthesize proteins cross-reactive with antigens of known helper-independent rat type C viruses.

Recently, a possibly analogous 30S defective retrovirus-like RNA (termed here 30S DRV RNA) has been discovered in murine leukemia viruses propagated in mouse cells (5, 9, 21; P. Besmer et al., personal communication). Unlike the 30S DRV RNA from rat cells, the 30S DRV RNA from mouse cells is not sequence related to a known murine type C retrovirus (9) and was originally detected as a 30S RNA species present in certain murine leukemia viruses in addition to their 30S genomic RNAs (5).

Other replication-defective exogenous viruses can be transmitted in pseudotype forms to a variety of cells. Although earlier attempts failed to transmit this endogenous information (1, 21), we initiated studies to transmit either a rat 30S subunit or a mouse 30S subunit from their RNA

forms as pseudotypes to heterologous cells. We report the successful transmission as pseudotypes of mouse and rat 30S subunits to heterologous cells and the isolation of a rat 30S subunit in a bat lung cell line free of the helper-independent type C virus used to form the pseudotype in the rat cells.

MATERIALS AND METHODS

Cells and viruses. The rat cells used in the present study included the following: HTP, a cell line derived from uterine carcinoma of a Sprague-Dawley rat; WR123, a cell line derived from a testicular tumor of a Fisher rat; and FRE clone 2, a contact-inhibited cell line derived from a Fisher rat (20). The mouse cells used included NIH cl 10, a thymidine kinase-deficient cell line derived from NIH 3T3 cells and obtained from Robert Goldberg, National Cancer Institute, and the SC-1 cell line (7), obtained from Janet Hartley, National Institute of Allergy and Infectious Diseases. The bat lung cell line Tblu, the dog thymus cell line Cf$_2$th, and the mink lung cell line CCL64 were obtained from the American Type Culture Collection.

The viruses used were: (i) ecotropic Moloney leukemia virus (Mo-MuLV), which was subjected to two cycles of terminal dilution on FRE rat cells; the virus was then used to infect NIH cl 10 cells to produce a culture chronically producing Mo-MuLV; (ii) Moloney mink cell focus-inducing virus, clone 83 (22), which was grown on the mink lung cell line; (iii) woolly monkey type C virus (WO-LV), which was subjected to two cycles of terminal dilution on the dog thymus cell line; the virus was then used to infect the HTP cell line to produce a culture chronically producing WO-LV; (iv) the M7 isolate of baboon type C virus (2), which was propagated on dog thymus cells after one cycle of terminal dilution on dog cells; this virus was obtained from Nara Battula, National Cancer Institute; and (v) Ki-MSV, which was produced as a feline pseudotype in mink lung cells; this culture has been described in detail previously (17, 23).

Preparation of cDNA. Tritiated cDNA probes were synthesized from endogenous reverse transcriptase reactions by methods previously described by utilizing calf thymus DNA fragments to obtain representative probes (24). The specific activity of all cDNA's was 2×10^7 cpm/μg, and all probes used protected at least 60% of their homologous ^{32}P-labeled RNAs at 1:1 molar ratios and 90% of these genomes at molar ratios of DNA to RNA of 4:1 to 5:1. The cDNA to detect Mo-MuLV was synthesized from Mo-MuLV clone 83 grown in mink lung cells. This virus is approximately 85% identical by oligonucleotide fingerprinting to the ecotropic Mo-MuLV used. To synthesize cDNA$_{Mo-MuLV}$, Mo-MuLV clone 83 was used rather than the ecotropic Mo-MuLV because it could be cloned and propagated in mink cells to avoid possible contamination with either mouse or rat endogenous retroviruses. The cDNA for the rat genetic sequences of Ki-MSV homologous to the rat 30S subunit has been described in detail (17, 23). The cDNA for the mouse 30S subunit expressed in NIH cl 10 cells was prepared as previously described (9). Briefly, the 30S RNA from the ecotropic Mo-MuLV grown in the NIH

cl 10 cell line was isolated on sucrose gradients and reverse transcribed with avian myeloblastosis virus reverse transcriptase and calf thymus fragments (9). The cDNA$_{mouse\ 30S}$ used in these studies is the same preparation used previously, and its characterization has been detailed earlier (9). The cDNA for WO-LV or baboon virus was prepared from each of these viruses grown in the dog thymus cell line as described above.

Preparation of RNAs. Total cellular RNA was prepared by phenol extraction as previously described (3), and viral 60 to 70S RNA was prepared by velocity sedimentation in 15 to 30% sucrose gradients from 1,000-fold-concentrated preparations of viruses banded twice by equilibrium sedimentation in sucrose gradients (3, 23).

Hybridization assays. Each hybridization reaction was incubated at 66°C for varying periods of time and contained, in 0.05 ml: 0.02 M Tris-hydrochloride, pH 7.5, 0.60 M sodium chloride, 0.1% sodium dodecyl sulfate, 5×10^{-4} M EDTA, approximately 2,500 cpm of [^{3}H]cDNA in trichloroacetic acid, and RNAs as indicated below. Reactions were incubated under mineral oil to avoid evaporation, and hybridization was assayed with S1 nuclease as previously described (3, 11). Hybridization kinetics were expressed as C$_r$t as suggested by Birnsteil (4).

RESULTS

Transmission of mouse 30S subunit. The virus complex released from the NIH cl 10 cells infected with ecotropic Mo-MuLV was filtered and used to infect either the contact-inhibited rat cell line FRE clone 2 or the rat tumor cell line WR123. These rat cells were chosen because ecotropic Mo-MuLV readily infected them and because in rat DNA under these stringent hybridization conditions, the cDNA$_{mouse\ 30S}$ hybridized to less than 5% of the input [^{3}H]cDNA. Thus, as in many of our earlier studies, we could distinguish endogenous sequences from potentially exogenously transmitted viral genes (19, 26). The cellular RNA from the infected rat cells and the 60 to 70S RNA in the viruses released from these cells were analyzed by molecular hybridization. The results are shown in Fig. 1 and 2. The 60 to 70S RNA from the Moloney virus released from the NIH cl 10 cells was analyzed with cDNA$_{mouse\ 30S}$ and cDNA$_{Mo-MuLV}$ (Fig. 1A). The kinetics of hybridization in which excess RNA was used indicate that the 60 to 70S RNA contains roughly equal concentrations of both Mo-MuLV RNA and 30S RNA. Because this culture produced a favorable ratio of helper-independent virus and the pseudotyped RNA, the Mo-MuLV was used to infect the heterologous rat cells. The RNA from FRE cells and FRE cells infected with this Moloney virus was hybridized to cDNA$_{mouse\ 30S}$ and cDNA$_{Mo-MuLV}$ (Fig. 1B). RNA homologous to Mo-MuLV was easily detected. Only a small amount of hybrid-
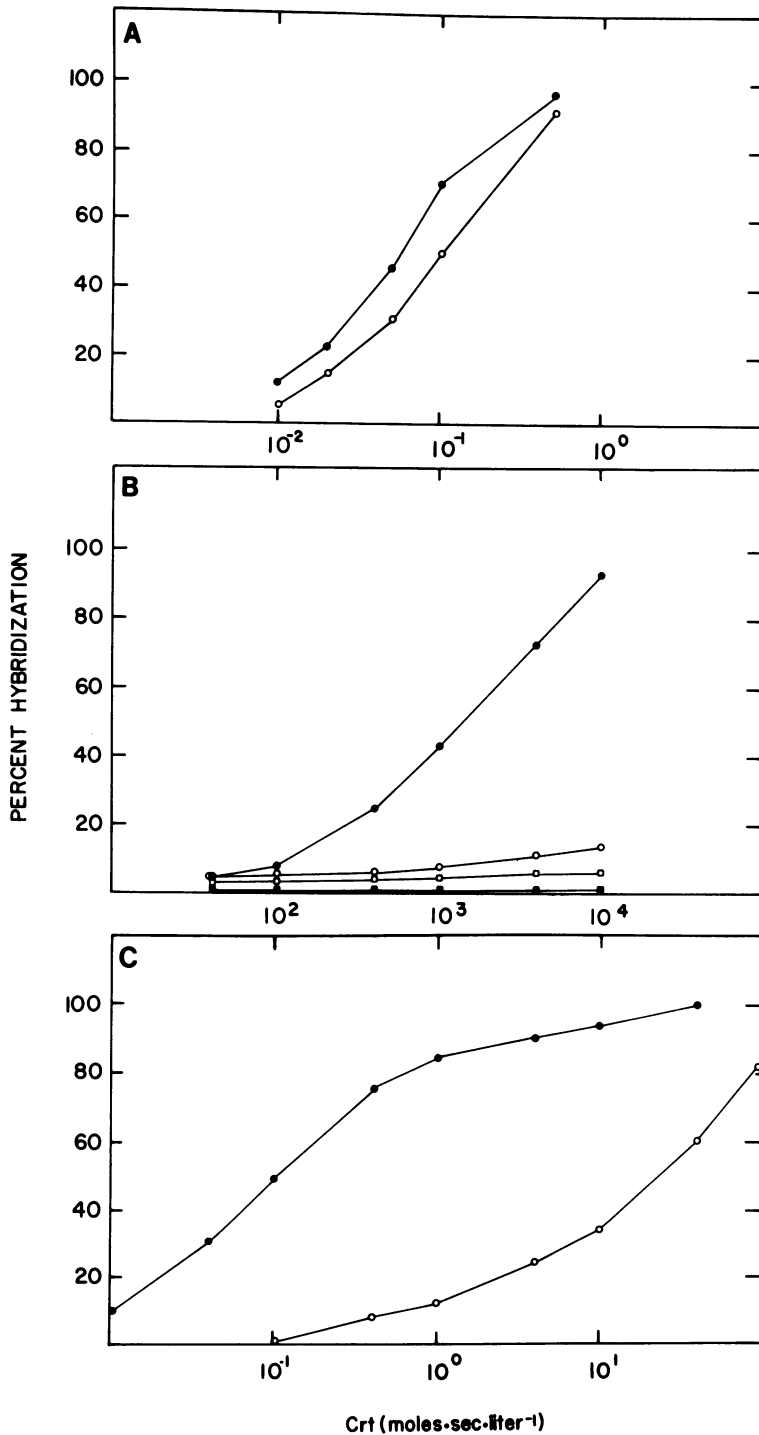
FIG. 1. *Transmission of mouse 30S subunit to contact-inhibited rat cells. The NIH cl 10 cell line producing ecotropic Mo-MuLV was grown to 90% confluence in 75-cm² Falcon flasks. At the time these cells reached confluence, 3 × 10⁴ FRE clone 2 rat cells were seeded in 35-mm petri dishes with 8 μg of polybrene per ml. An 8-ml amount of fresh medium was added to the mouse culture, and an 18-h yield of virus was collected and filtered through 0.45-μm membrane filters (Millipore Corp.). The rat cells were then infected with the undiluted*

ization was achieved with cDNA$_{mouse\ 30S}$ when the infected rat cell RNA was used; at a C$_r$t of 1 $\times$ 10^4 to 2 $\times$ 10^4 mol·s·liter^{-1} only 16 to 18% of the cDNA$_{mouse\ 30S}$ was protected. To more convincingly demonstrate the RNA sequences homologous to the cDNA$_{mouse\ 30S}$, 60 to 70S viral
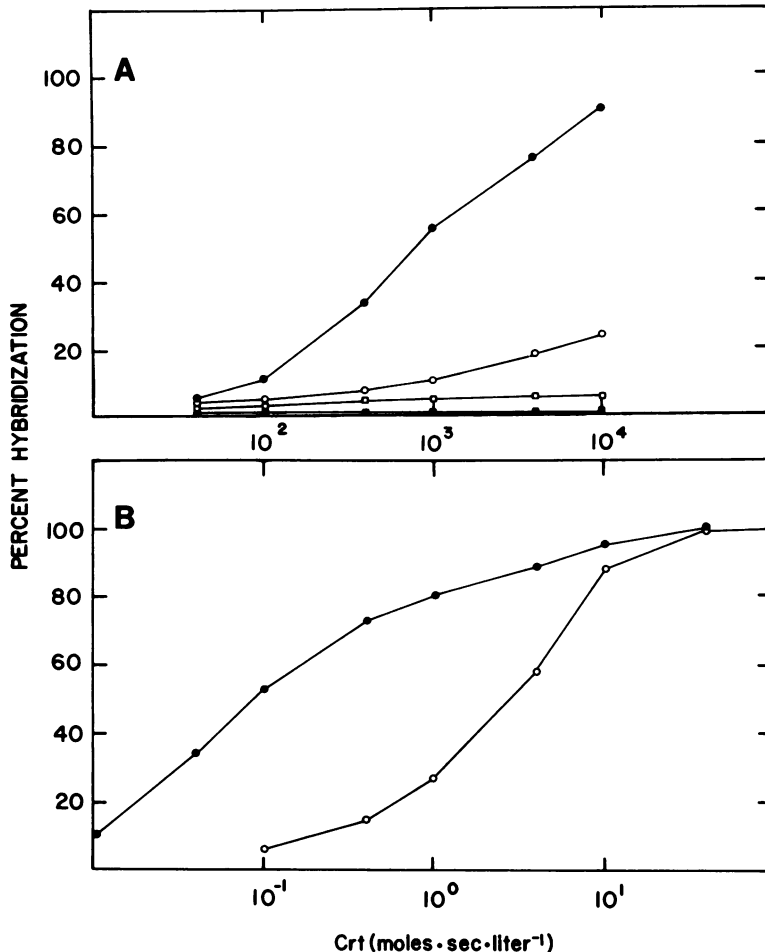

FIG. 2. *Transmission of mouse 30S subunit to transformed rat cells. WR123 tumor cells were infected with the virus released from NIH cl 10 cells producing Mo-MuLV, and total cellular RNA and 60 to 70S RNA were prepared from the cultures as described in the legend to Fig. 1. The cDNA's and the conditions of assay were the same as those described in the legend to Fig. 1. (A) Hybridization of total cellular RNA from uninfected WR123 cells to cDNA$_{Mo-MuLV}$ (■) and cDNA$_{mouse\ 30S}$ (□), and hybridization of total cellular RNA from infected WR123 cells to cDNA$_{Mo-MuLV}$ (●) and cDNA$_{mouse\ 30S}$ (○). (B) Hybridization of 60 to 70S RNA from virions released from infected WR123 cells to cDNA$_{Mo-MuLV}$ (●) and cDNA$_{mouse\ 30S}$ (○).*

virus. When the rat cells reached confluence in approximately 3 to 4 days, they were subcultured to 150-cm^2 flasks. Approximately 1 week later, some cells were harvested, and total cellular RNA was prepared as described in the text. Parallel flasks were further subcultured, and 60 to 70S viral RNA was prepared from virions released from these cells from approximately 3 liters of supernatant fluid as described in the text. The various RNAs shown below were hybridized to a cDNA$_{Mo-MuLV}$ and cDNA$_{mouse\ 30S}$ as described in the text. Each hybridization contained approximately 2,500 cpm of cDNA in trichloroacetic acid, and background in the absence of added RNA was less than 40 cpm. (A) Hybridization of 60 to 70S RNA from Mo-MuLV released from NIH cl 10 mouse cells to cDNA$_{Mo-MuLV}$ (●) and cDNA$_{mouse\ 30S}$ (○). (B) Hybridization of total cellular RNA from uninfected FRE clone 2 cells to cDNA$_{Mo-MuLV}$ (■) and cDNA$_{mouse\ 30S}$ (□), and hybridization of total cellular RNA from FRE clone 2 cells infected with the virus released from NIH cl 10 cells to cDNA$_{Mo-MuLV}$ (●) and cDNA$_{mouse\ 30S}$ (○). (C) Hybridization of 60 to 70S RNA from FRE cells infected as described above with Mo-MuLV from NIH cl 10 mouse cells to cDNA$_{Mo-MuLV}$ (●) and cDNA$_{mouse\ 30S}$ (○).

RNA was prepared from the viruses released from the infected FRE cells. The results are shown in Fig. 1C. Again, sequences homologous to Mo-MuLV were readily detected. The mouse 30S-related sequences were also detected, but 50% hybridization was not achieved until $C_r t$ values were approximately 4×10^1 mol·s·liter^{-1}. At a $C_r t$ value of 10^2 mol·s·liter^{-1}, 80% of the cDNA was protected.

To achieve higher levels of mouse 30S sequences in heterologous cells, a similar transmission of the mouse genes to rat cells was performed by using transformed rat cells as the recipient cell line. The results are shown in Fig. 2A and B. Once again, RNA homologous to the Mo-MuLV cDNA was readily detected. The mouse 30S-related sequences were detected somewhat more easily, and at a $C_r t$ value of 10^4 mol·s·liter^{-1}, approximately 24% of the cDNA$_{mouse\ 30S}$ was protected. The 60 to 70S RNA released from the infected WR123 cells was also tested for hybridization to cDNA$_{mouse\ 30S}$. A hybridization value of 50% was achieved at a $C_r t$ of 5 mol·s·liter^{-1}, and 100% hybridization to the cDNA$_{mouse\ 30S}$ was possible. As a further control, ecotropic Mo-MuLV was terminally diluted onto WR123 cells at a 10^{-5} dilution of the original virus stock. The virus was allowed to spread in the culture, and 60 to 70S RNA was prepared from the virus released. Less than 3% hybridization was detected to the cDNA$_{mouse\ 30S}$ with comparable concentrations of this 60 to 70S RNA of Moloney virus grown in this way in WR123 cells. The results indicate that mouse 30S sequences can be transmitted to rat cells by using Mo-MuLV as a helper virus.

Transmission of a rat 30S subunit to bat cells. Transmission of endogenous rat genes homologous to the rat portions of Ki-MSV and Ha-MSV was performed by using the helper-independent virus isolated from a woolly monkey. The 60 to 70S RNA from HTP rat cells producing WO-LV was analyzed for its content of rat 30S RNA and WO-LV RNA, and the results are shown in Fig. 3A. The cDNA$_{rat\ 30S}$ and cDNA$_{WO-LV}$ hybridize at approximately equal rates to this RNA, and thus this culture was chosen for transmission experiments. Bat lung cells were infected with the virus released from HTP cells producing WO-LV, and the RNA was analyzed by molecular hybridization. The results (Fig. 3B) indicate that both WO-LV RNA and rat 30S subunit RNA can be detected in bat cells. As in the studies on mouse 30S RNA, we could detect only low levels of rat 30S subunit RNA in the heterologous cells. Therefore, we analyzed the 60 to 70S RNA released in virions from the infected bat cells. The results (Fig. 3C)

show that the cDNA$_{rat\ 30S}$ could be completely protected by the 60 to 70S RNA released from the bat cell line, although the concentration of 30S related RNA in this viral RNA was low. The results indicate that a rat 30S subunit could also be transmitted to a heterologous cell by using a WO-LV pseudotype.

Isolation of bat nonproducer cells. The experiments described above demonstrated that mouse and rat 30S subunits can be pseudotyped and transmitted to heterologous cells. The levels of RNA expressed in the heterologous cells were low in each case, and the ratio of helper virus RNA to 30S RNA was approximately 100:1 in virions released from the heterologous cells. Although transmission could be demonstrated, we could not rule out the possibility that transmission had occurred by recombination between the helper virus and the pseudotyped 30S RNA. To show that a 30S subunit could be transmitted to a heterologous cell free of replicating helper virus, bat cells were infected with the rat 30S (WO-LV) pseudotype released from HTP cells, and single-cell bat clones were isolated 4 h after the infection was initiated. A series of such single-cell clones was subcultured, and the supernatant from these cells was assayed for WO-LV by reverse transcriptase assays. Of approximately 50 clones, 25 were found to produce WO-LV by this test. All but two were discarded. The remaining clones were analyzed for their viral RNA content after a portion of the cells was saved (Table 1). Four clones (clones 1, 2, 21, and 23) had low but detectable levels of RNA homologous to the cDNA$_{rat\ 30S}$. None of these clones released WO-LV viral particles as judged by reverse transcriptase assays on supernatants of such cells. One of the four clones (clone 21) was found to be producing WO-LV RNA but not WO-LV reverse transcriptase. Thus, three clones (clones 1, 2, and 23) were candidates for bat cells infected with a rat 30S subunit free of replicating WO-LV.

Clone 1 was further analyzed (Fig. 4). The RNAs from uninfected bat cells and bat cell clone 1 were hybridized to cDNA$_{WO-LV}$ or cDNA$_{rat\ 30S}$ as shown in Fig. 4A. No hybridization to either cDNA was detected with RNA from uninfected bat cells. RNA from bat clone 1 hybridized appreciably to cDNA$_{rat\ 30S}$, with 50% hybridization achieved at a $C_r t$ value of 1 $\times 10^4$ to 2 $\times 10^4$ mol·s·liter^{-1}. No hybridization was detected to cDNA$_{WO-LV}$ with RNA from this cell line, and no hybridization (<2%) was detected with this RNA to a cDNA$_{3'}$ synthesized from polyadenylic acid-purified WO-LV genomic RNA (data not shown).

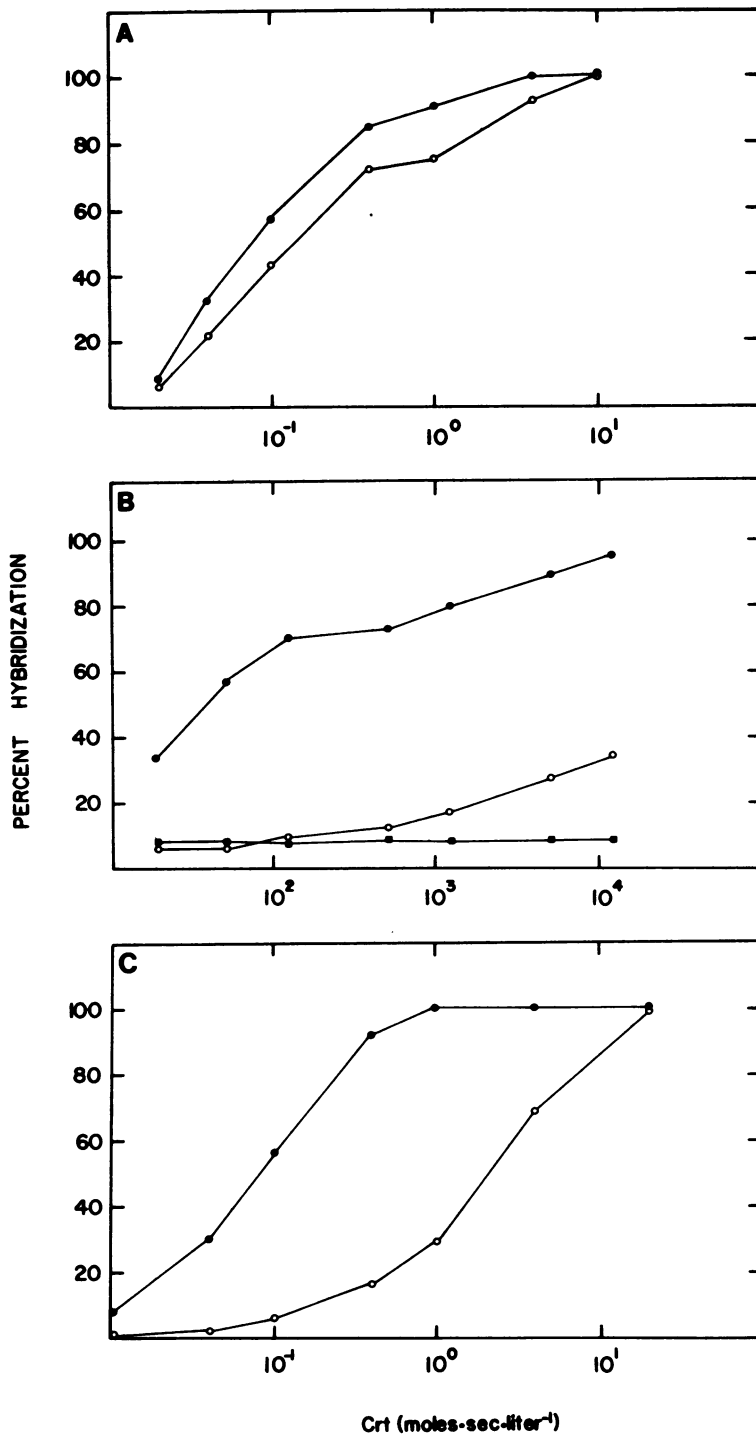Bat clone 1 was infected with a different

FIG. 3. *Transmission of rat 30S subunit to bat lung cells. The HTP cell line infected with woolly type C virus was grown to 90% confluence in 75-cm² Falcon flasks. At this time, 3 × 10⁴ bat lung cells were seeded in 35-mm petri dishes containing a solution with 4 µg of polybrene per ml. An 8-ml amount of fresh medium was added to the rat cells, and an 18-h yield of virus was collected and filtered through a 0.45-µm Millipore filter. This virus was used to infect the bat cells, and the cells were subcultured as described in the legend to Fig. 1. Total cellular and viral RNA were prepared as described in the text and in the legend to Fig. 1. cDNA$_{rat\ 30S}$ was prepared from Ki-MSV as previously described (18), and cDNA$_{WO-LV}$ was prepared from WO-LV terminally diluted on a dog thymus cell line (Cf₂th). Each hybridization assay contained approximately 2,500 cpm of cDNA in trichloroacetic acid, and background in the absence of RNA was less than 60 cpm. (A) Hybridization of 60 to 70S RNA from WO-LV grown in HTP cells to cDNA$_{WO-LV}$ (●) and cDNA$_{rat\ 30S}$ (○). (B) Hybridization of total cellular RNA from bat cells infected as described above to cDNA$_{WO-LV}$ (●) and cDNA$_{rat\ 30S}$ (○), and hybridization of total cellular RNA from uninfected bat cells to either cDNA (■). (C) Hybridization of 70S RNA from bat cells infected with WO-LV as described above to cDNA$_{WO-LV}$ (●) and cDNA$_{rat\ 30S}$ (○).*

helper-independent type C virus, the M7 isolate of baboon virus. The RNA in putative viral particles released from bat clone 1 or particles released from bat clone 1 infected with M7 virus was analyzed by molecular hybridization, and the results are shown in Fig. 4B. The hybridi-

TABLE 1. *Analysis of bat cell clones for WO-LV and rat 30S subunit[a]*

Bat cell clone	Amt of polymerase (cpm incorporated)	% Hybridization at a $C_r t$ of 4,000 mol·s·liter^{-1} with:	
		cDNA$_{WO-LV}$	cDNA$_{Rat\ 30S}$
1	1,385	<5	47
2	755	<5	24
3	910		<5
5	2,915		<5
8	1,420		<5
10	2,105		<5
12	918		<5
16	2,110		<5
18	1,140		<5
20	1,450		<5
21	2,015	91	17
22	970		<5
23	1,680	<5	19
25	1,170		<5
27	2,005		<5
28	1,270	90	<5
29	1,885		<5
30	1,035		<5
31	1,825		<5
32	805,865	86	36
33	1,170		<5
34	600,530	95	24
36	2,053		<5

[a] A total of 3×10^4 bat lung cells were seeded in 35-mm Falcon plates in media containing 4 μg of polybrene per ml. A 24-h collection of virus was prepared from the HTP cell line releasing woolly type C virus as described in the legend to Fig. 1. The virus was diluted twofold with medium and filtered through a 0.45-μm filter, and the bat cells were infected. After 4 h, the cells were cloned in Falcon microtest II plates. When the clones had grown to confluence within each well, random clones were subcultured to 35-mm plates and assigned consecutive numbers. Each clone was subcultured to two 150-cm^2 flasks, and the supernatant fluid was tested for reverse transcriptase by using polyriboadenylate:oligodeoxythymidylate$_{12-18}$ as template. Of the 50 clones tested, 27 produced virus by this test, and only two of them (clones 32 and 34) were saved. A portion of each of the rest of the clones was viably frozen, and total cellular RNA was prepared from the rest of the cells. Each RNA was then hybridized to cDNA$_{rat\ 30S}$, and the indicated RNAs were also hybridized to cDNA$_{WO-LV}$. Polymerase is expressed as counts per minute of [^{3}H]TMP incorporated in trichloroacetic acid in 0.01 ml of a 100-fold concentrate of culture fluid. The specific activity of the [^{3}H]TMP was 30 Ci/mmol; assay conditions have been detailed previously (3, 4).

zation kinetics are expressed as a function of $V_0 t$ (14). Bat clone 1 cells released no detectable rat 30S RNA, but bat clone 1 cells infected with M7 virus released both M7 RNA and rat 30S RNA. Bat cells infected with M7 virus released no rat 30S RNA. In other studies 96% hybridization of cDNA$_{rat\ 30S}$ could be achieved with 60 to 70S RNA prepared from the M7–bat clone 1 culture at $C_r t$ values of 1×10^{-1} to 2×10^{-1} mol·s·liter^{-1}. No hybridization (<3%) could be detected to cDNA$_{WO-LV}$ with the same viral RNA, even at $C_r t$ values of 10 mol·s·liter^{-1}. The results indicate that a rat 30S subunit can be transmitted as a pseudotype to a heterologous nonproducer cell line and can be expressed in the heterologous cells free of the replicating helper type C virus used to perform the transmission. In other studies, we extracted total cellular DNA from the bat clone 1 cell line and detected the rat 30S sequences but failed to detect WO-LV sequences. No sequences in uninfected bat cell DNA were detected (less than 2% hybridization) with the cDNA$_{rat\ 30S}$. We have not yet determined whether the rat 30S DNA is integrated into the bat cell DNA.

DISCUSSION

The current experiments demonstrate the transmissibility of a mouse and rat 30S subunit. By rescuing each 30S subunit with a helper-independent type C virus, we have transmitted a mouse 30S subunit to a heterologous rat cell line and a rat 30S subunit to a heterologous bat cell line. In addition, using approaches similar to those used for the isolation of spleen focus-forming virus nonproducer cells (26), we have isolated a rat 30S subunit in a bat nonproducer cell line free of replicating helper-independent type C virus. In prior studies, we have emphasized the similarities between the endogenous rat 30S subunits and various exogenous replication-defective type C viruses (12, 16). The transmissibility of an endogenous rat 30S subunit to heterologous nonproducer cells further extends the similarity between the 30S subunit and other replication-defective retroviruses.

The evidence that a 30S subunit can be pseudotyped and transmitted may provide the data with which to interpret why 30S subunit-related genes are reiterated in the DNA of normal rats. Perhaps in the course of evolution, helper-independent rat type C viruses have pseudotyped 30S RNAs, reverse transcribed these RNAs, and reinserted the DNA copies into various sites in the rat genome. Such reiteration could occur by interactions between helper-independent rat viruses and 30S subunits similar to those that occurred in the WO-LV transmission of a 30S
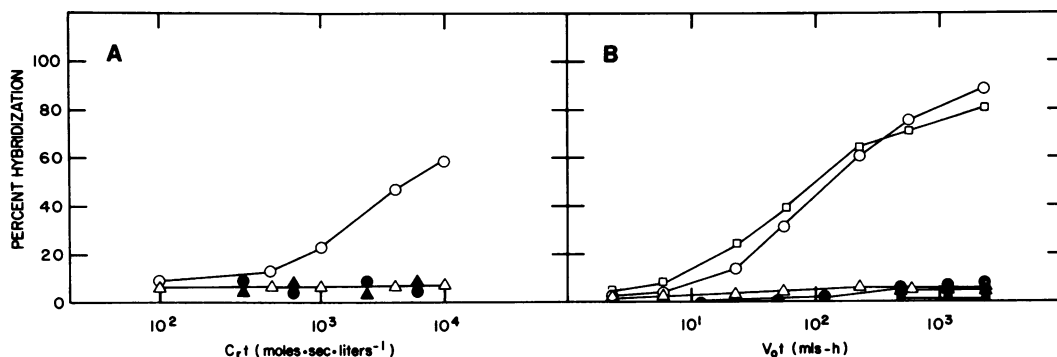
FIG. 4. *Analysis of bat nonproducer cells. (A) Total cellular RNA was extracted from uninfected bat cells and bat clone 1. The RNA was hybridized either to cDNA$_{rat\ 30S}$ or cDNA$_{WO-LV}$. Symbols: O, hybridization of cDNA$_{rat\ 30S}$ to RNA from bat clone 1; △, hybridization of cDNA$_{rat\ 30S}$ to RNA from uninfected bat cells; ●, hybridization of cDNA$_{WO-LV}$ to RNA from bat clone 1; ▲, hybridization of cDNA$_{WO-LV}$ to RNA from uninfected bat cells. (B) Bat clone 1 cells or uninfected bat cells were infected with the M7 isolate of baboon type C virus grown originally in dog thymus cells. Supernatant fluid was collected at 24-h intervals from comparable numbers of the two bat cells in 150-cm^2 flasks and stored at 4°C until 300 ml was obtained. The fluid was clarified by centrifugation at 10,000 × g for 15 min and then sedimented at 100,000 × g for 2 h to concentrate the virus, and the putative RNA was then extracted (14). The deproteinized RNA was hybridized to the cDNA's indicated below, and hybridization kinetics were calculated as a function of V$_0$t (milliliters × hours) as suggested by Ringold et al. (14). Each assay contained approximately 2,500 cpm of either cDNA$_{rat\ 30S}$ or cDNA$_{M7}$ in trichloroacetic acid. Hybridization of supernatant of M7-infected bat clone 1 cells to cDNA$_{M7}$ (□) and cDNA$_{rat\ 30S}$ (O); hybridization of supernatant of M7-infected bat cells to cDNA$_{rat\ 30S}$ (△); hybridization of supernatant of uninfected bat clone 1 cells to cDNA$_{rat\ 30S}$ (●) and cDNA$_{M7}$ (■).*

subunit to heterologous cells. Such a model would be consistent with the protovirus hypothesis as described by Temin (25). The transmissibility of 30S subunits as pseudotypes allows further predictions of this theory to be tested because restriction maps can now be defined on a variety of biologically cloned genomes, and comparisons of the genomic organization and expression of different 30S subunits in the DNA and RNA of various normal and transformed rat cells can be made.

The observation that a 30S subunit is transmissible also suggests the need to describe carefully the pedigrees of helper-independent type C viruses. Clearly potential endogenous retrovirus-like sequences can be pseudotyped and transmitted to other cells and then contaminate helper-independent viruses in subtle ways. The need to terminally dilute helper-independent viruses when they are passaged from one cell line to another would seem to be emphasized.

As well as providing information about the biology of an endogenous 30S rat subunit, the current studies provide further ways by which to compare a rat 30S subunit with Ki-MSV. Both replication-defective genomes can be transmitted to bat cells. Ki-MSV-related RNA is expressed at levels almost two orders of magnitude higher in bat cells than the levels of rat 30S subunit RNA (unpublished data). The major structural differences between Ki-MSV and the 30S subunit are the composition of the 5' and 3' ends of the two viruses (23). Ki-MSV has mouse sequences at its 5' and 3' ends which are derived from a helper-independent mouse type C virus (23). Thus, it is tempting to speculate that the difference in the ends of the two genomes determines their relative ability to be expressed in these heterologous cells, either by determining an integration site which facilitates transcription or by the ends themselves providing transcriptional signals.

The function of the 30S DRV RNAs in mouse and rat cells is not known, and cells expressing those RNAs and releasing them as viral pseudotypes do not appear morphologically transformed in culture. In the case of rat 30S DRV RNA, this is surprising in view of its close relationship to Ha-MSV and Ki-MSV RNAs. However, the relatively low levels of rat 30S DRV RNA in rat cells and in particular in the bat cells investigated here preclude a definitive interpretation of the unchanged morphology of these cells because morphological transformation may depend on a critical threshhold concentration of 30S DRV RNA that was not reached in the cells studied here. It is also conceivable that the oncogenicity in animals of certain mouse and rat (NRK cells cause carcinomas if inoculated into rats [V. Klemert, personal communication]) cell lines considered untransformed in cells in culture may be related to the presence and concen-

tration of 30S DRV RNA; 30S DRV RNA could function directly in neoplastic transformation in the animal or could become active only by recombination with some other viral or cellular gene.

The bat nonproducer cell line should provide a useful tool with which to examine further the biology of the rat endogenous 30S-related genes. For the first time, this rat endogenous gene has been biologically cloned. Perhaps similar approaches will also allow biological cloning of ecotropic helper-independent rat type C viruses in heterologous cells. Such biologically purified helper-independent rat type C viruses and replication-defective rat 30S subunits would greatly facilitate further molecular studies on both Ki-MSV and other sarcoma viruses derived from rats (13).

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